CLAIMS

1. A method for identifying an agent which alters the interaction between a protein tyrosine phosphatase and a tyrosine phosphorylated polypeptide which is a substrate of the protein tyrosine phosphatase, comprising:

(a) contacting in the absence and in the presence of a candidate agent, a substrate trapping mutant of a protein tyrosine phosphatase and a detectably labeled tyrosine phosphorylated peptide which is a substrate of the protein tyrosine phosphatase under conditions and for a time sufficient to permit formation of a complex between the tyrosine phosphorylated peptide and the substrate trapping mutant protein tyrosine phosphatase, wherein the substrate is capable of generating a fluorescence energy signal; and

(b) comparing the fluorescence energy signal level in the absence of the agent to the fluorescence energy signal level in the presence of the agent, wherein a difference in the fluorescence energy signal level indicates the agent alters formation of a complex between the protein tyrosine phosphatase and the substrate.

2. The method of claim 1 wherein the fluorescence energy signal is a fluorescent polarization signal.

3. The method of claim 1 wherein the detectably labeled tyrosine phosphorylated peptide comprises a fluorophore.

4. The method of claim 3 wherein the fluorophore is selected from the group consisting of fluorescein, rhodamine, Texas Red, AlexaFluor-594, AlexaFluor-488, Oregon Green, BODIPY-FL and Cy-5.

5. The method of claim 1 wherein the substrate comprises a polypeptide sequence derived from a protein selected from the group consisting of VCP, $p130^{cas}$, EGF receptor, p210 bcr:abl, MAP kinase, Shc, insulin receptor, lck and T cell receptor zeta chain.

6. The method of claim 1 wherein the substrate trapping mutant protein tyrosine phosphatase comprises a protein tyrosine phosphatase in which the wildtype protein tyrosine phosphatase catalytic domain invariant aspartate residue is replaced with an amino acid which does not cause significant alteration of the Km of the enzyme but which results in a reduction in Kcat to less than 1 per minute.

7. The method of claim 1 wherein the substrate trapping mutant protein tyrosine phosphatase comprises a protein tyrosine phosphatase in which the wildtype protein tyrosine phosphatase catalytic domain is mutated at an amino acid position occupied by a cysteine residue.

8. The method of claim 6 wherein the substrate trapping mutant protein tyrosine phosphatase comprises a protein tyrosine phosphatase in which at least one wildtype tyrosine residue is replaced with an amino acid that is not capable of being phosphorylated.

9. The method of claim 8 wherein at least one wildtype tyrosine residue is replaced with an amino acid selected from the group consisting of alanine, cysteine, aspartic acid, glutamine, glutamic acid, phenylalanine, glycine, histidine, isoleucine, lysine, leucine, methionine, asparagine, proline, arginine, valine and tryptophan.

10. The method of claim 8 wherein at least one tyrosine residue that is located in a protein tyrosine phosphatase catalytic domain is replaced.

11. The method of claim 8 wherein at least one tyrosine residue that is located in a protein tyrosine phosphatase active site is replaced.

12. The method of claim 8 wherein the wildtype tyrosine residue is replaced with phenylalanine.

13. The method of claim 8 wherein the wildtype tyrosine residue that is replaced is a protein tyrosine phosphatase conserved residue.

14. The method of claim 13 wherein the conserved residue corresponds to tyrosine at amino acid position 676 in human PTPH1.

15. The method of claim 8 wherein at least one tyrosine residue is replaced with an amino acid that stabilizes a complex comprising the protein tyrosine phosphatase and at least one substrate molecule.

16. The method of claim 8 wherein the substrate trapping mutant protein tyrosine phosphatase is a mutated protein tyrosine phosphatase selected from the group consisting of PTP1B, PTP-PEST, PTPγ, MKP-1, DEP-1, PTPμ, PTPX1, PTPX10, SHP2, PTP-PEZ, PTP-MEG1, LC-PTP, TC-PTP, CD45, LAR and PTPH1.

17. A method for identifying an agent which alters the interaction between a protein tyrosine phosphatase and a tyrosine phosphorylated polypeptide which is a substrate of the protein tyrosine phosphatase, comprising:

(a) contacting, in the absence and in the presence of a candidate agent, a protein tyrosine phosphatase and a detectably labeled tyrosine phosphorylated peptide which is a substrate of the protein tyrosine phosphatase under conditions and for a time sufficient to permit

dephosphorylation of the substrate by the protein tyrosine phosphatase, wherein the substrate is capable of generating a fluorescence energy signal;

(b) exposing the protein tyrosine phosphatase and the substrate to a reaction terminator molecule and thereby terminating dephosphorylation of the substrate; and

(c) comparing the fluorescence energy signal level of substrate which remains phosphorylated in the absence of the agent to the energy signal level of substrate which remains phosphorylated in the presence of the agent, wherein a difference in the fluorescence energy signal level indicates the agent alters the interaction between the protein tyrosine phosphatase and the substrate.

18. A method of identifying an agent which alters the interaction between a protein tyrosine phosphatase and a tyrosine phosphorylated polypeptide which is a substrate of the protein tyrosine phosphatase, comprising:

(a) contacting, in the absence and in the presence of a candidate agent, a protein tyrosine phosphatase and a detectably labeled tyrosine phosphorylated peptide which is a substrate of the protein tyrosine phosphatase under conditions and for a time sufficient to permit dephosphorylation of the substrate by the protein tyrosine phosphatase, wherein the substrate is capable of generating a fluorescence energy signal;

(b) exposing the protein tyrosine phosphatase and the substrate to a reaction terminator molecule and thereby terminating dephosphorylation of the substrate; and

(c) comparing the fluorescence energy signal level of substrate which is dephosphorylated in the absence of the agent to the energy signal level of substrate which is dephosphorylated in the presence of the agent, wherein a difference in the fluorescence energy signal level indicates the agent alters the interaction between the protein tyrosine phosphatase and the substrate.

19. The method of either claim 17 or claim 18 wherein the fluorescence energy signal level is a fluorescence polarization signal level.

20. The method of either claim 17 or claim 18 wherein the detectably labeled tyrosine phosphorylated peptide substrate and the reaction terminator molecule comprise an energy transfer molecule donor-acceptor pair, and wherein the fluorescence energy signal level is a fluorescence resonance energy transfer level.

21. The method of claim 20 wherein the detectably labeled tyrosine phosphorylated peptide substrate comprises an energy transfer acceptor molecule and the reaction terminator molecule comprises an energy transfer donor molecule.

22. The method of claim 20 wherein the detectably labeled tyrosine phosphorylated peptide substrate comprises an energy transfer donor molecule and the reaction terminator molecule comprises an energy transfer acceptor molecule.

23. The method of either claim 17 or claim 18, wherein the reaction terminator molecule is an antibody specific for phosphotyrosine and wherein the step of exposing is subsequent to the step of contacting the PTP and the substrate.

24. The method of either claim 17 or claim 18, wherein the reaction terminator molecule is selected from the group consisting of an antibody specific for a PTP substrate, an antibody specific for a PTP catalytic domain, a substrate trapping mutant protein tyrosine phosphatase, vanadate, an SH2 domain polypeptide, an IRS1 PTB domain polypeptide, an shc PH domain polypeptide and a non-antibody molecule that specifically binds to a tyrosine phosphorylated form of the detectable PTP substrate.

25. The method of claim 24 wherein the molecule that specifically binds to a tyrosine phosphorylated form of the detectable substrate is selected from the group consisting of an SH2 domain polypeptide and a PTP-PID domain polypeptide.

26. A method for determining dephosphorylation of a substrate by a protein tyrosine phosphatase, comprising:

contacting a protein tyrosine phosphatase and a detectable substrate of said protein tyrosine phosphatase under conditions and for a time sufficient to permit dephosphorylation of said detectable substrate by said protein tyrosine phosphatase to form a reaction mixture, wherein the detectable substrate comprises a detectably labeled tyrosine phosphorylated peptide which is a substrate of the protein tyrosine phosphatase:

exposing to said reaction mixture a reaction terminator molecule; and

determining the level of tyrosine phosphorylated substrate in the reaction mixture by detecting anisotropic motion of the substrate, and therefrom determining dephosphorylation of the detectable substrate by the protein tyrosine phosphatase.

27. A method for identifying an agent that regulates dephosphorylation of a detectable substrate by a protein tyrosine phosphatase, comprising:

contacting a protein tyrosine phosphatase and a detectable substrate of said protein tyrosine phosphatase under conditions and for a time sufficient to permit dephosphorylation of said detectable substrate by said protein tyrosine phosphatase in the presence of a candidate agent to form a first incomplete reaction mixture, and in the absence of a candidate agent to form a second incomplete reaction mixture, wherein the detectable substrate comprises a detectably labeled tyrosine phosphorylated peptide which is a substrate of the protein tyrosine phosphatase:

exposing to each of said first and second incomplete reaction mixtures a reaction terminator molecule to form a first complete reaction mixture and a second complete reaction mixture: and

comparing the level of tyrosine phosphorylated substrate in each of said first and second complete reaction mixtures by detecting anisotropic motion of the substrate, and therefrom identifying an agent that regulates dephosphorylation of the detectable substrate by the protein tyrosine phosphatase.

28. The method of either claim 26 or claim 27 wherein the reaction terminator molecule is an antibody specific for phosphotyrosine and wherein the step of exposing is subsequent to the step of contacting the PTP and the substrate.

29. The method of either claim 26 or claim 27 wherein the reaction terminator molecule is selected from the group consisting of an antibody specific for a PTP substrate, an antibody specific for a PTP catalytic domain, a substrate trapping mutant protein tyrosine phosphatase, vanadate, an SH2 domain polypeptide, an IRS1 PTB domain polypeptide, an she PH domain polypeptide and a non-antibody molecule that specifically binds to a tyrosine phosphorylated form of the detectable PTP substrate.

30. The method of claim 29 wherein the molecule that specifically binds to a tyrosine phosphorylated form of the detectable substrate is selected from the group consisting of an SH2 domain polypeptide and a PTP-PID domain polypeptide.

31. The method of either claim 26 or claim 27 wherein the detectable substrate comprises a fluorophore and wherein anisotropic motion is detected by fluorescence polarization.

32. The method of claim 31 wherein the fluorophore is selected from the group consisting of fluorescein, rhodamine, Texas Red, AlexaFluor-594, AlexaFluor-488, Oregon Green, BODIPY-FL and Cy-5.

33. The method of either claim 26 or claim 27 wherein the substrate comprises a polypeptide sequence derived from a protein selected from the group consisting of VCP, $p130^{cas}$, EGF receptor, p210 bcr:abl, MAP kinase, She, insulin receptor, lck and T cell receptor zeta chain.